STIC-ILL

RC 845. H45

From: Scheiner, Laurie
Sent: Wednesday, February 21, 2001 8:09 PM
To: STIC-ILL
Subject: reference request (09/407,432)

Mamiya et al., Hepatology, (Oct., 1998) Vol. 28, No. 4 PART 2, pp.462A. ISSN: 0270-9139. Thanks!

Laurie Scheiner
Art Unit 1648
CM1 8E05
308-1122

1197 INHIBITION OF A CELLULAR DEAD-BOX RNA HELICASE BY HEPATITIS C VIRUS CORE PROTEIN. N Mamiya and HJ Worman. Departments of Medicine and of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY.

Hepatitis C virus (HCV) encodes its own RNA helicase that unwinds the viral RNA, which is not capped at its 5' end and uses internal ribosome entry sites. This suggests that mechanisms may have evolved to surpress the translation of capped cellular mRNAs and allow viral RNA to preferentially gain access to the ribosomes. In a yeast two-hybrid screen of 8 x 10^6 recombinant clones of a human hepatocyte cDNA library with the cytoplasmic domain of HCV core protein, we isolated three positive interactors that encoded portions of the DEAD-box protein DBX. A glutathione-S-transferse fusion protein containing a portion of DBX interacted with the cytoplasmic domain of HCV core protein synthesized by *in vitro* transcription-translation. Strong *in vitro* binding between these two proteins still occurred in 1 M NaCl or 1% NP40. When expressed in cultured cells with epitope tags, DBX was found to be in a mostly diffuse distribution in the cytoplasm and core protein in a more aggregated pattern in the endoplasmic reticulum co-localized with a significant portion of DBX. DBX is the human orthologue of mouse PL10 and previous studies have shown that PL10 can functionally replace the essential yeast DEAD-box ATP-dependent RNA helicase Ded1p. In the yeast two-hybrid assay, HCV core protein interacted with mouse PL10, which is 98% identical in sequence to DBX, but not with yeast Ded1p, which is only 54% identical. We were able to complement the lethal Ded1 mutation of yeast with human DBX. Expression of HCV core protein had no effect on the growth of wild-type yeast, however, it virtually abolished the growth of DBX and PL10 complemented Ded1⁻ strains. The cytoplasmic domain of HCV core protein did not inhibit growth as significantly as the full-length protein, suggesting that a "trapping" mechanism at the endoplasmic reticulum membrane, as opposed to just binding, is responsible for inhibition of DBX activity. These results demonstrate that HCV core protein inhibits a DEAD-box RNA helicase. This may be a mechanism that enables HCV RNA to preferentially gain access to cell ribosomes by inhibiting host cell RNA translation. It may lead to the death of hepatocytes that express high levels of HCV structural proteins. As DBX binds to HCV core protein under very stringent conditions, inhibitors of viral assembly may also be designed based upon the sequence of DBX. [Supported by a grant from the Blowitz-Ridgeway Foundation.]

1198 **SEVERITY OF CHRONIC HEPATITIS C, IRON OVERLOAD, AND MUTATIONS IN THE HEMOCHROMATOSIS GENE (HFE)**

M Sampietro, R Boldorini*, A Casatta, M Amato, N Corbetta, D Tavazzi, A Pellagatti, R Nunziata*, M Mattioli, M Del Vitto, V Molteni, AL Fracanzani, S Fargion, G Fiorelli. Dipartimento di Medicina Interna, IRCCS Ospedale Maggiore - Università di Milano; *Dipartimento di Scienze Mediche, Novara - Università di Torino, Italy

A relationship between iron status and the activity of chronic hepatitis C has been proposed. Iron overload is frequent in patients with hepatitis C, and iron depletion may induce a reduction of transaminases that is not related to a modification of the viral load. A role for the hemochromatosis (GH) gene in the iron overload of hepatitis C has been hypothesized, supported from studies of HLA-A alleles frequency. HFE, a candidate gene for GH was recently identified, allowing a direct investigation of its relationship with iron overload and with the histopathological features of hepatitis C. We studied 53 naive male patients (age 47±13y) with a histological diagnosis of chronic hepatitis C. The two mutations of HFE (C282Y and H63A) were detected by restriction analysis after PCR amplification of trace DNA extracted from serum. Liver histology was evaluated using the modified activity index (HAI) (Ishak, 1995) and histological iron distribution was scored as described by Deugnier (1993). Liver iron concentration (LIC) was determined by atomic spectrophotometry absorption. Mild or moderate iron overload was observed in 16/53 (30%) patients. Iron overload was present in 52% of 21 patients with HAI >3 vs.15% in 32 patients with HAI <3 (p=0.006). Overall 9/16 (56%) patients with iron overload carried HFE mutations: the frequency of C282Y, the major GH mutation, was only marginally increased while H63D had an allelic frequency of 0.25 in HC patients with iron overload vs. 0.081 in patients with normal iron status (p=0.02) and 0.128 in 128 normal controls. The score for hepatocytic iron was increased in patients carrying H63D (p<0.05). Our findings confirm that iron status is associated with the histological severity of hepatitis C. The HFE genotype, as a major determinant of the iron status, may be one of the factors of the genetic background able to influence the host response to HCV and the clinical evolution of chronic infection.

1199 **DOES HIV INFECTION AGGRAVE CHRONIC HEPATITIS C IN IV DRUG USERS ? A CASE-CONTROL STUDY**

L Serfaty[1], D Costagliola[2], D Wendum[3] O Picard[4], MC Meyohas[5], PM Girard[6], J Lebas[4], D . C Delamare[7], R Poupon[1], C Housset[1]. [1]Service d'Hépato-gastroentérologie, [2]INSERM SC4, Services de [3]Anatomopathologie, [4]Médecine Interne, [5]Maladies Infectieuses, [7]Bactériologie-Virologie, Hôpital St-Antoine; [6]Service de Maladies Infectieuses, Hôpital Rothschild, Paris, France.

The influence of HIV infection on the natural course of HCV infection is still poorly defined because of numerous confounding factors. We therefore undertook a case-control study in order to compare chronic hepatitis C with or without HIV infection in patients contaminated via IV drug use.

Methods : Between 1993 and 1997, we prospectively included 39 patients with HIV infection (cases) who responded to the following criteria: positive anti-HCV ELISA 2, elevated ALT activity, IV drug use as risk factor, level of CD4>350/ml, no AIDS and no anti-retroviral treatment. Among 626 consecutive patients with chronic hepatitis C and without HIV infection referred to our department during the same period, 39 (controls) were paired to cases according to age, sex, IV drug use as risk factor and duration of HCV infection. All patients had serum HCV RNA quantitation (b DNA), HCV genotyping (Inno Lipa) and liver biopsy. Liver biopsy specimens were blindly readressed by the same pathologist.

Results : Demographic characteristics of cases and controls were as following : age 36±6 yrs, sex ratio 2.9, HCV infection duration 14±4 yrs. The majority of patients were infected by genotypes 1 (45% vs 60%) and 3 (38% vs 33%) in cases and controls. The level of viremia was significantly higher in cases than in controls (15.9±13.1 vs 11.5±10.8, p=0.02). Knodell score was significantly higher in cases than in controls (9.7±3.8 vs 7.3±3.5, p=0.008). According to the METAVIR histological grading, the cases exhibited higher activity (2.2±0.8 vs 1.6±0.7, p<0.0001) and fibrosis (1.8±1 vs 1.4±0.7, p=0.05) than the controls. There was no significant correlation between level of viremia and histological activity in both groups. The percentage of cirrhosis tended to be higher in cases (10% vs 2.5%).

Conclusions : Using a case-control study, we could demonstrate that in IV drug users with chronic hepatitis C, HIV infection leads to higher HCV viremia and more severe liver injury.

1200 HCV VIREMIA AFTER LIVER TRANSPLANTATION FOR HCV CIRRHOSIS: RELATION TO TYPE OF IMMUNOSUPPRESSION. GV Papatheodoridis, D Andrew*, G Clewley*, G Dusheiko, B Davidson, K Rolles, and AK Burroughs. Liver Transplantation Unit, *Dept. of Virology, Royal Free Hospital, London, UK.

The effect of immunosuppression on HCV viremia and natural history of HCV recurrence after liver transplantation (OLT) for HCV cirrhosis has not been clarified. We evaluated the fluctuation of HCV viremia after OLT and its relation to type of immunosuppression in a cohort of 37 of 63 HCV transplant patients who survived at least 12 mos and had available serial serum samples. Serum HCV RNA levels were determined by a 2nd generation bDNA assay (Chiron) pre-transplant and at 1 week (1-wk), 2 weeks (2-wk), 3 months (3-mo) and 12 months (12-mo) after OLT. Initial immunosuppression was combination [cyclosporin (CYA) or tacrolimus (TACR) and prednisolone (PRED) with (n=26) or without (n=4) azathioprine (AZA)] in 30 (group I) and monotherapy (CYA or TACR) in 11 patients (group II). In Group I, PRED was withdrawn in a mean of 6.3±2.7(2-12) mos after OLT. Median serum HCV RNA levels (x10^6genomes/mL) were pre-transplant:0.2[a], 1-wk:0.3[b], 2-wk:0.5[c], 3-mo:7.0[d] and 12-mo:8.0[e] (P for: a or b vs d or e<10^{-3}, c vs d=0.03, c vs e=0.002). At 1-wk and 2-wk, HCV RNA levels were not associated with pre-transplant HCV RNA level, type of initial immunosuppression, acute rejection episodes or treatment. At 3-mo, HCV RNA levels were significantly lower in Group I than in Group II (median: 4.6 vs 69.0, P=0.003). However, HCV RNA decreased significantly between 3-mo and 12-mo in the monotherapy group (from a median of 69.0 to 4.9, P=0.018), whereas it did not change or decrease in Group I (from a median of 4.6 to 8.4, P=0.28). At 12-mo, HCV RNA levels were significantly correlated with previous duration of PRED treatment overall (r=0.42, P=0.01) as well as in Group I alone (r=0.48, P=0.007). The correlation between PRED duration and 12-mo HCV RNA was independent of primary immunosuppression (CYA or TACR), previous courses of anti-rejection treatment and occurrence or timing of acute hepatitis. In conclusion, less initial immunosuppression appears to be associated with higher viremia levels at 3-mos after transplant. However at 12-mo, higher HCV RNA levels are significantly associated with longer duration of steroid treatment.